# Host Plant Quality Factors That Influence the Growth and Development of *Oxyops vitiosa*, a Biological Control Agent of *Melaleuca quinquenervia*

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The Australian weevil *Oxyops vitiosa* was released in 1997 in Florida as a biological control agent of *Melaleuca quinquenervia.* The larvae of this agent are flush-feeders, found only on the growing tips of their host. Knowledge of this restriction to feeding on the growing tips and other nutritional requirements may assist in the establishment and dispersal of this species. Therefore, *O. vitiosa* survival was assessed when neonates were fed *M. quinquenervia* leaves from branches that had dormant buds or emerging bud leaves. Additionally, the influence of leaf quality from different sites and within sites was determined by the feeding of neonates emerging bud leaves collected at three sites and from three leaf qualities (poor, intermediate, and high). Within-site leaf qualities were described in the field by leaf color and in the laboratory by percentage dry mass and nitrogen. Larval survival was lowest when fed leaves from branches that had dormant buds. Associated with this low survival were high leaf toughness and percentage dry mass. When larvae were fed emerging bud leaves, most of the variation in larval survival and performance was attributed to differences in within-site plant quality. Generally, the highest-quality leaves had relatively low percentage dry mass and high percentage nitrogen. Larval survival generally decreased when fed the poor-quality leaves, and in one site, the intermediate-quality leaves. Larvae required less time to develop to adults when fed the high-quality leaves. Development time increased in females but not in males when the larvae were fed the poor-quality leaves. Adult biomass of both females and males generally increased when the larvae were fed the high-quality leaves from two of the three sites. The results indicate that the larvae of *O. vitiosa* are restricted to feeding on flush foliage with low toughness. Additionally, variations in foliar percentage dry mass and nitrogen influence larval survival and performance. This knowledge benefited the development of mass-production nursery sites and the selection of suitable release sites, which facilitated the establishment of this biological control agent.

## INTRODUCTION

Biological control efforts against the Australian melaleuca tree *Melaleuca quinquenervia* (Cav.) Blake (Myrtaceae) have resulted in the 1997 release in south Florida of the leaf-feeding weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) (Center *et al.,* 2000). Both the adults and the larvae of this species have been observed feeding primarily on flush-foliage on tip leaves of the melaleuca tree (Purcell and Balciunas, 1994). In south Florida, the tree produces this flush-growth generally in the winter and spring from the apical buds after flowering (Van *et al.,* 2001). This insect is most abundant in Australia on *M. quinquenervia* trees that are growing rapidly, often from suckers or landscaped plants (Purcell and Balciunas, 1994). This suggests that larval survival, growth, and development may be influenced by the host nutritional quality. Thus, a better understanding of the nutritional requirements of *O. vitiosa* would aid establishment efforts of this species in Florida for biological control of *M. quinquenervia.*

The melaleuca tree is an aggressive weed threatening the biodiversity of the south Florida everglades ecosystem (Turner *et al.,* 1998). Originally from eastern Australia, this tree was introduced to south Florida around the turn of the century by horticulturists (Morton, 1966). Following its introduction, the tree has spread through the natural areas of south Florida that include, and are adjacent to, the Everglades National Park and the Big Cypress Preserve. With the dramatic decreases in water flows of south Florida for flood control to protect agriculture and urban areas, this species and many other exotic weeds have flourished. The tree now occupies more than 200,000 ha in the region and,

because of its ability to resprout from periodic freezes, has the potential to invade coastal wetlands of southern Louisiana and eastern Texas (Turner *et al.,* 1998).

The nutrition of herbivores feeding on tree foliage may be limited by several key factors, among them moisture and nitrogen content (Mattson and Scriber, 1987). Compared with herbaceous plants, tree foliage typically has lower levels of water, nitrogen, and mineral elements (Mattson and Scriber, 1987). Additionally, fiber content and leaf toughness may be important factors limiting the survival, growth, and development of tree-feeding folivores (Coley and Barone, 1996). Consequently, the herbivores that feed on tree foliage typically have lowered performance and conversion efficiencies compared with herb-feeders (Scriber and Feeny, 1979; Mattson, 1980). The goals of this study were to quantify select plant quality factors and to determine their impact on the survival and performance of the *M. quinquenervia* biological control agent *O. vitiosa.*

## MATERIALS AND METHODS

### Preliminary Test

*Plant quality.* A preliminary test was conducted to investigate the degree of dependence of *O. vitiosa* neonates on flush-foliage. This was determined by the recording of *O. vitiosa* neonate survival when fed *M. quinquenervia* branches with and without emerging bud leaves. Branches were collected at Tree Tops Park (TT), Broward Co., Florida during spring 1997. The branches without emerging leaves had dormant buds and were characterized as stage 1 and those with leaves that were fully emerged from the bud were characterized as stage 4 (T. K. Van *et al.,* USDA/ARS, Ft. Lauderdale, FL, unpublished data). The leaves were analyzed for leaf toughness ($n = 10$) with a modified gram gauge (Wheeler and Center, 1996). Leaf toughness was estimated for 15 consecutive leaves from the apical tip toward the base of the branch. All statistical analyses were conducted on SAS/PC (SAS Institute, 1990). To determine whether leaf toughness changed with leaf position on the stem, the data were analyzed with linear regression. To determine whether leaf toughness differed between the branches with dormant buds and those from emerging buds, the regression coefficients were compared with analysis of covariance (ANCOVA). Percentage dry mass of leaves was determined gravimetrically ($n = 16$) by the combining of all the leaves in each branch and the comparison of the mass of leaves weighed fresh and after drying at 60°C for 48 h.

*Larval survival.* Neonates ($n = 40$) were fed leaves from freshly collected *M. quinquenervia* branches until they reached the adult stage. Foliage and larvae were kept in petri dishes ($15 \times 2$ cm) lined with moistened filter paper and sealed with parafilm to retain moisture. The filter paper was moistened, the frass was removed, and the *M. quinquenervia* branches were replaced about every 3 days. Larvae were reared at 27°C, 90% RH, and L14:D10 h photophase. Data were collected on larval survival to the prepupal, pupal, and adult stages.

### Second Test—Among- and Within-Sites

*Plant quality.* Subsequent collections included branches that had buds with emerging leaves (stage 4 buds; T. K. Van *et al.,* unpublished data) collected from three south Florida sites located at Holiday Park (HP), Tree Tops Park, both Broward Co., and Krome Avenue and Highway 27 (Krome), Dade Co., Florida. All collections were conducted during the spring of 1997. At each site, leaves were classified into one of three categories based upon leaf color, which may be a useful predictor of relative plant quality and overall suitability of select herbivores. Leaves were classified by the chromatic colors green or yellow with the notation of G or Y (or an intermediate of the two, e.g., GY), respectively, based upon the Munsell color chart (Anonymous, 1977). Each color may be further subdivided with numbers that refer to the darkness of the hue, where dark is symbolized as 0/ and light as 10/, and the degree of its saturation, where the greater the value, symbolized as /10, the greater its saturation. The leaves collected at sites were classified as poor (5Y 8/8 to 5GY 7/8), intermediate (5GY 5/6 to 7.5GY 4/4), and high (5GY 4/6 to 7.5GY 3/4).

Percentage dry mass of leaves collected at the three sites and three categories within each site were determined gravimetrically by leaf position ($n = 16$), zero being the youngest leaf at the branch tip. The percentage dry mass was determined as described previously for individual leaves. Additionally, leaves were digested with a modified Kjeldahl method (Hach *et al.,* 1987) and percentage nitrogen content was determined ($n = 16$) by an ammonia-selective ion method (Greenberg *et al.,* 1992). Standard reference tomato leaves (National Institute of Standards, Gaithersburg, MD) were analyzed as controls and the values were adjusted for percentage recovery. To determine whether leaf percentage dry mass or nitrogen differed among sites and qualities within sites, a two-way ANCOVA was performed, where leaf position served as the covariate. The means were compared with the Ryan's Q multiple comparison test ($P = 0.05$). To determine differences in elevations and slopes of percentage dry mass or nitrogen over leaf positions on a branch within each site, the regression coefficients were compared with ANCOVA.

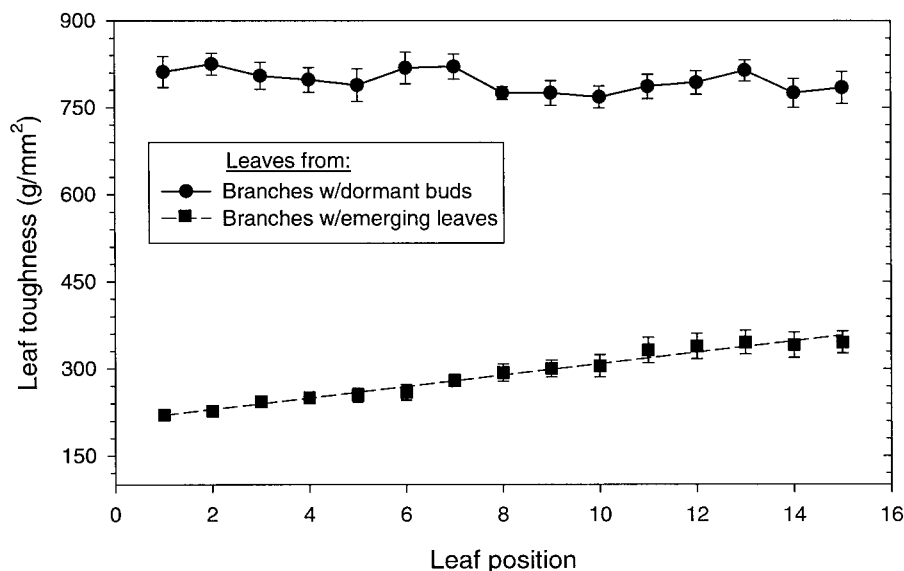*Larval performance.* Neonates ($n = 40$) were fed

**FIG. 1.** Mean toughness (± SE) of *M. quinquenervia* leaves located on branches with dormant buds and on branches with emerging bud leaves. Leaf toughness was significantly greater for leaves from branches with dormant buds (regression not significant) than for leaves from branches with emerging bud leaves (y = 209.9 + 9.9 x; $r^2$ = 0.45; $P <$ 0.0001).

leaves individually in petri dishes as described above until they developed to the adult stage. Data were collected on several performance parameters including larval survival, biomass gain, and development time to the adult stage. To determine whether site, quality, or adult sex significantly influenced these insect performance parameters, a three-way ANOVA was performed.

<div align="center">

**RESULTS**

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*Preliminary Test*

*Leaf toughness.* Leaf toughness was significantly greater for leaves from branches with dormant buds compared with those from branches that had buds with emerging leaves ($F_{1,218}$ = 27.78; $P <$ 0.0001; Fig. 1). Toughness of the leaves from dormant buds ranged from 750 to nearly 800 g/mm$^2$, whereas that of the leaves from branches with emerging bud leaves ranged from 200 to $<$350 g/mm$^2$. The leaf toughness from branches with dormant buds did not change with leaf position ($P >$ 0.09) in contrast to those with emerging bud leaves, which increased toward the branch base. Dry mass of the leaves from branches with dormant buds (43.8 ± 0.7%) was significantly greater ($F_{1,45}$ = 685.02; $P <$ 0.0001) than that of the leaves from branches with emerging bud leaves (23.2 ± 0.5%).

*Larval survival.* Survival of neonates to the prepupal, pupal, and adult stages was significantly reduced when fed leaves from branches with dormant buds ($F_{1,10}$ = 41.63; $P <$ 0.0001). Only 7.5 (± 4.8%) of the

neonates survived to the prepupal, pupal, and adult stages when fed leaves from branches with dormant buds compared with 47.5 (±3.7%) for those fed leaves from branches that had emerging bud leaves.

*Second Test—Among- and Within-Sites*

*Plant quality.* Percentage dry mass of leaves was influenced by site ($F_{2,2143}$ = 26.52; $P <$ 0.0001), quality ($F_{2,2143}$ = 459.59; $P <$ 0.0001), leaf position (covariate) on the branch ($F_{1,2143}$ = 241.70; $P <$ 0.0001), and interaction of site and quality ($F_{4,2143}$ = 225.14; $P <$ 0.0001). Among-site comparisons indicated that percentage dry mass of leaves was greatest for poor-quality leaves collected at the TT site followed by those collected at the Krome and HP sites ($F_{2,717}$ = 191.31; $P <$ 0.0001; Fig. 2). Leaves of the intermediate quality had the greatest percentage dry mass from the HP and Krome sites ($F_{2,717}$ = 60.64; $P <$ 0.0001). The high-quality leaves had the greatest percentage dry mass at the Krome site, followed by the HP site, which was greater than that collected at the TT site ($F_{2,710}$ = 140.60; $P <$ 0.0001). Additionally, within-site analyses indicated that percentage dry mass of leaves was greatest for the poor-quality leaves at the TT ($F_{2,716}$ = 1369.11; $P <$ 0.0001) and Krome ($F_{2,716}$ = 93.63; $P <$ 0.0001) sites and greatest for the high- and poor-quality leaves at the HP site ($F_{2,712}$ = 9.36; $P <$ 0.0001).

Percentage dry mass of leaves decreased significantly with distance from the tip for all site × quality combinations except for the high-quality branches, which either had positive slopes or had slopes that
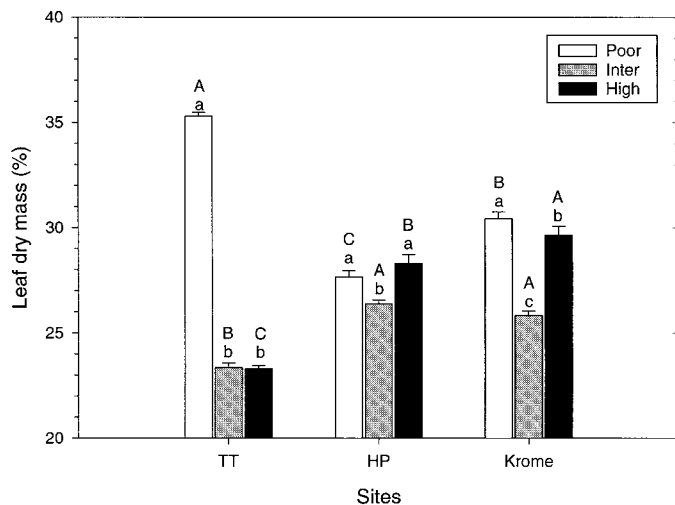
**FIG. 2.** Mean percentage dry mass (± SE) of *M. quinquenervia* leaves from three sites and three categories of plant quality from each site. Bars with the same uppercase letters within a plant quality were not significantly different and bars with the same lowercase letters within a site were not significantly different, both according to a Ryan's Q mean comparison test ($P < 0.05$).

were not significantly different from zero (Table 1; Fig. 3). Moreover, the slopes of the lines for high quality were greater than those of the intermediate quality, which were generally greater than, or equal to (e.g., Krome), those of poor quality. Additionally, for each site the elevations of the lines for poor-quality leaves were significantly greater (Table 1) than those for intermediate leaves, which were greater than, or equal to (e.g., Krome), those for high-quality leaves.

Given the observed differences in percentage dry mass of the leaves among sites, leaf quality, and leaf position, the nutrients available to herbivores would be diluted differently according to these three parameters. Therefore, herein, nitrogen content is expressed on a fresh-mass basis. Percentage nitrogen of leaves was influenced by site ($F_{2,119} = 99.40$; $P < 0.0001$), quality ($F_{2,119} = 15.75$; $P < 0.0001$), leaf position (covariate) on the branch ($F_{1,119} = 25.49$; $P < 0.0001$), and interaction of site and quality ($F_{4,119} = 3.92$; $P = 0.0050$; Fig. 4). Percentage nitrogen of foliage of all three quality levels (high: $F_{2,40} = 40.24$; $P < 0.0001$; intermediate: $F_{2,41} = 46.96$; $P < 0.0001$; and poor: $F_{2,39} = 22.57$; $P < 0.0001$) was highest when collected at the Krome site, followed by that of the HP and TT sites (Fig. 4). Furthermore, within-site analyses indicated that the percentage nitrogen was greatest for the high-quality leaves, followed by that of the intermediate- and poor-quality leaves at the HP ($F_{2,45} = 5.78$; $P = 0.0058$) and TT ($F_{2,42} = 27.32$; $P < 0.0001$) sites, whereas, no differences occurred among the leaf qualities from Krome (Fig. 4).

Foliar percentage nitrogen was distributed differently from the branch tip to the base according to the quality of branch collected. Percentage nitrogen of leaves decreased significantly with distance from the tip for all site × quality combinations except for the high-quality branches, which all increased toward the branch base (Table 2; Fig. 5). The slopes of the lines for high quality were significantly greater than those of the poor and intermediate qualities at all sites. Additionally, the elevations of the lines for the intermediate- and poor-quality branches from HP and Krome were generally greater than those for the high-quality leaves (Table 2).

*Insect survival.* Neonate survival to the prepupal stage decreased significantly when fed the poor-quality leaves at the TT and HP sites and the intermediate-

## TABLE 1

Regression Coefficients for Percentage Dry Mass of *M. quinquenervia* Leaves Categorized into Three Qualities from Three Sites in Southeastern Florida

| Site | Quality | Coefficients | | $r^2$ | $P$ |
| | | $B_0$[a] | $B_1$[b] | | |
|---|---|---|---|---|---|
| TT | High | 24.9 c | −0.1 a | 0.24 | 0.0565 |
| | Intermediate | 27.9 b | −0.6 b | 0.95 | <0.0001 |
| | Poor | 35.5 a | −0.02 c | 0.04 | 0.4748 |
| HP | High | 25.0 c | 0.4 a | 0.79 | <0.0001 |
| | Intermediate | 29.1 b | −0.4 b | 0.76 | <0.0001 |
| | Poor | 33.3 a | −0.8 c | 0.86 | <0.0001 |
| Krome | High | 29.2 b | 0.07 a | 0.21 | 0.0716 |
| | Intermediate | 29.7 b | −0.5 b | 0.79 | <0.0001 |
| | Poor | 34.9 a | −0.6 b | 0.77 | <0.0001 |

[a] Elevation coefficients within a site followed by the same letter were not significantly different according to ANCOVA and a linear contrast procedure.

[b] Slope coefficients within a site followed by the same letter were not significantly different according to ANCOVA and a linear contrast procedure.
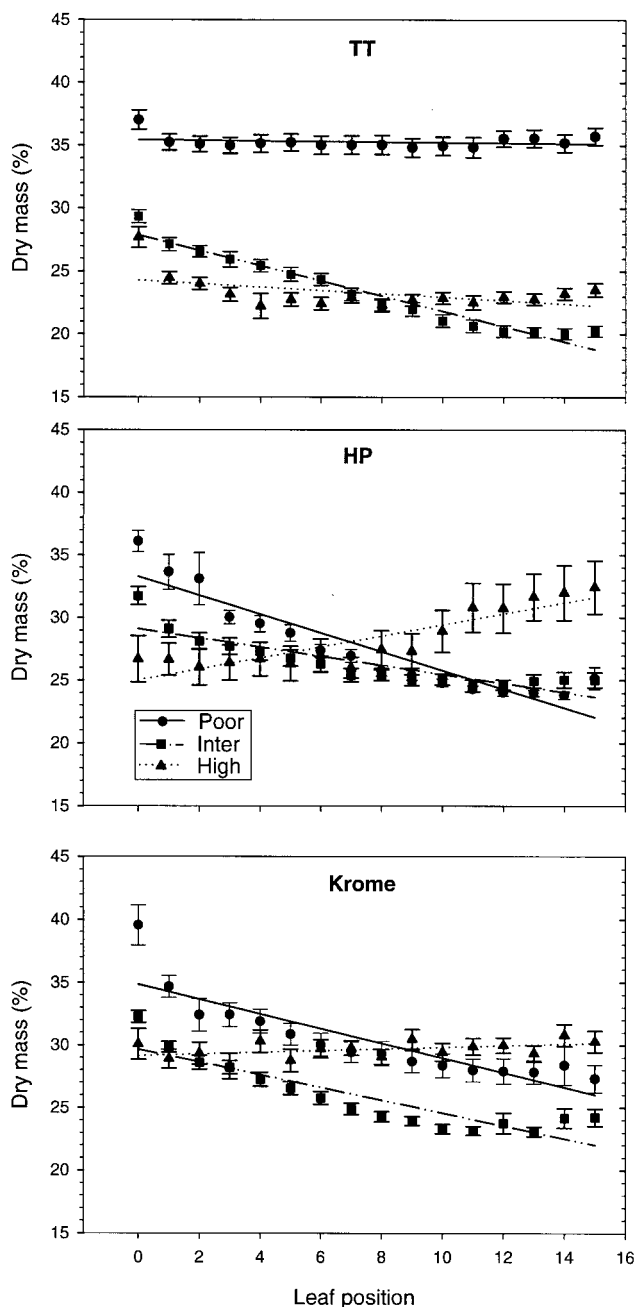
**FIG. 3.** Mean percentage dry mass (± SE) of *M. quinquenervia* leaves at different leaf positions from the tip. Leaves were from three sites and three categories of plant quality from each site. Percentage dry mass of leaves decreased in the poor-quality (see Table 1 for coefficients and probabilities) and intermediate-quality leaves and increased in the high-quality leaves from the branch tip toward its base.

quality leaves at the TT site (Fig. 6). However, regardless of within-site quality differences, overall first instar survival differed little when fed leaves from different sites ($F_{2,33} = 0.85$; $P > 0.4$).

*Insect growth and development.* Development time

to the adult stage was significantly affected by both site ($F_{2,183} = 17.19$; $P < 0.0001$) and quality ($F_{2,183} = 10.90$; $P < 0.0001$) and the interaction of these two factors ($F_{4,183} = 6.94$; $P < 0.0001$; Fig. 7A). Among-site comparisons indicated that adult development time was significantly shortest when fed the intermediate-quality leaves from the HP site ($F_{2,60} = 27.74$; $P < 0.0001$) compared with the same quality of leaves from the other two sites. Little difference among sites occurred in adult development time for the other quality categories of leaves. Within-site comparisons indicated that development time was generally shorter for larvae fed the high-quality leaves from the TT ($F_{2,65} = 13.42$; $P < 0.0001$) and the Krome ($F_{2,70} = 10.08$; $P < 0.0001$) sites compared with those fed leaves from other plant quality categories. Adult development time was also influenced by the interaction of insect sex and plant quality ($F_{2,183} = 3.93$; $P = 0.0214$). However, only female adult development time ($F_{2,106} = 7.07$; $P = 0.0013$) was greater when fed the poor-quality leaves (35.7 ± 1.1 days) compared with those fed the intermediate-quality (33.1 ± 0.7 days) and high-quality (32.1 ± 0.4 days) leaves. Male development time was not influenced by leaf quality (32.6 ± 0.3 days; $P > 0.05$).

Adult biomass was also significantly affected by site ($F_{2,185} = 5.72$; $P = 0.0039$), plant quality ($F_{2,185} = 3.43$; $P = 0.0346$), and their interaction ($F_{4,185} = 4.38$; $P = 0.0021$; Fig. 7B). Little difference occurred in adult biomass among sites; however, it was greatest for larvae fed the intermediate-quality leaves from the HP and Krome sites ($F_{2,61} = 5.00$; $P = 0.0097$) compared with the same-quality leaves from the TT site.
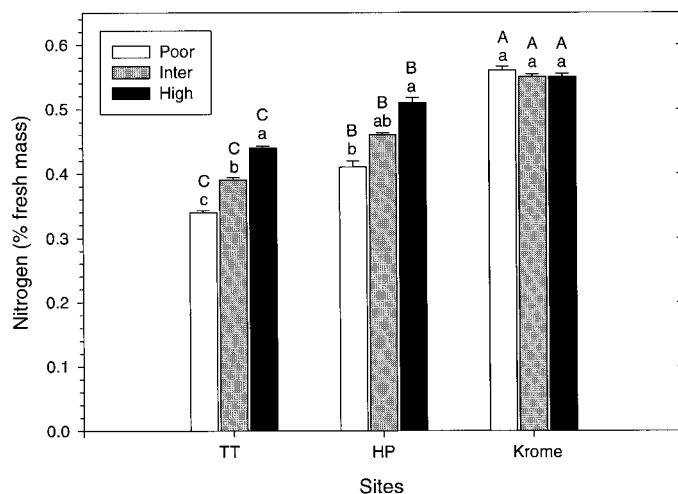


**FIG. 4.** Mean percentage nitrogen (fresh mass ± SE) of *M. quinquenervia* leaves collected from three sites of three categories of plant quality. Bars with the same uppercase letters within a plant quality category were not significantly different and bars with the same lowercase letters within a site were not significantly different, both according to a Ryan's Q mean comparison test ($P < 0.05$).

## TABLE 2

Regression Coefficients for Percentage Nitrogen (Fresh Mass) of *M. quinquenervia* Leaves Categorized
into Three Qualities from Three Sites in Southeastern Florida

| Site | Quality | Coefficients | | $r^2$ | $P$ |
| | | $B_0{}^a$ | $B_1{}^b$ | | |
|------|---------|------|------|------|------|
| TT | High | 0.42 b | 0.002 a | 0.29 | 0.0399 |
| | Intermediate | 0.48 a | −0.011 c | 0.92 | <0.0001 |
| | Poor | 0.37 c | −0.004 b | 0.30 | 0.0296 |
| HP | High | 0.44 c | 0.010 a | 0.85 | <0.0001 |
| | Intermediate | 0.52 b | −0.008 b | 0.90 | <0.0001 |
| | Poor | 0.60 a | −0.026 c | 0.89 | <0.0001 |
| Krome | High | 0.50 c | 0.005 a | 0.74 | 0.0003 |
| | Intermediate | 0.62 a | −0.008 c | 0.89 | <0.0001 |
| | Poor | 0.58 b | −0.002 b | 0.06 | 0.4839 |

[a] Elevation coefficients within a site followed by the same letter were not significantly different according to ANCOVA and a linear contrast procedure.
[b] Slope coefficients within a site followed by the same letter were not significantly different according to ANCOVA and a linear contrast procedure.

Within-site analyses indicated that adult biomass values were significantly greatest ($F_{2,67}$ = 8.72; $P$ = 0.0004) when larvae were fed the high-quality leaves from the TT site compared with the other quality categories of leaves from the same site. Additionally, larvae fed the intermediate-quality leaves from the Krome site had greater adult biomass ($F_{2,70}$ = 2.83; $P$ = 0.0661) than larvae fed the poor-quality leaves from the same site. Insect sex also influenced adult biomass. The females had significantly greater biomass (41.8 ± 0.4 mg) than the males (36.0 ± 0.5 mg; $F_{1,185}$ = 71.38; $P$ < 0.0001). However, none of the interactions were significant, suggesting that the effects of site and quality influenced the male and female biomasses similarly.

## DISCUSSION

These results suggest that both within- and among-site variations in the percentage of dry mass and nitrogen of *M. quinquenervia* leaves influenced the survival, growth, and development of *O. vitiosa* larvae. Additionally, the most dramatic effect was found in the low larval survival when they were feeding on leaves from branches with dormant buds. Associated with this low larval survival were high leaf toughness and percentage dry mass. However, when larvae were fed leaves that were emerging from buds, most of the variation in larval survival and performance could be directly related to within-site plant quality differences, namely, percentage dry mass and nitrogen of the leaves. Although larval survival and adult biomass were similar in larvae fed leaves from different sites, development time for larvae fed the intermediate- and poor-quality leaves from HP was generally shortest.

Other site factors, not investigated here, may influence *O. vitiosa* survival, such as hydroperiod, natural enemies, and secondary plant chemistry. This species completes larval development on leaves in the tree canopy and then drops or climbs down to the ground where it excavates a pupal cell in the soil (Purcell and Balciunas, 1994). *M. quinquenervia* occupies coastal wetlands in Australia (Turner *et al.,* 1998), and many sites infested with this tree species in south Florida are flooded for extensive periods (Center *et al.,* 2000). The prepuae of *O. vitiosa* are not expected to survive long periods in water, and weevil populations have not established at sites in Florida with long hydroperiods (Center *et al.,* 2000). Additionally, natural enemies may be important regulators of *O. vitiosa* populations in their native range; however, in Florida generalist predators have been observed attacking *O. vitiosa* larvae only in a few localities, including the predacious pentatomid *Podisus mucronatus* Uhler (P. Pratt, USDA/ARS, Ft. Lauderdale, FL, personal communication). Other factors that potentially may impact herbivore populations are the numerous foliar terpenoids of *M. quinquenervia.* These terpenoids have been well documented in Australia (Brophy *et al.,* 1989) and in other countries (e.g., Ramanoelina *et al.,* 1994); however, little is known about their composition in Florida. Even less is known about the biological relevance of *M. quinquenervia* terpenoids to the associated herbivores, including this biological control agent. Different sites in Australia and Florida may be dominated by distinct *M. quinquenervia* chemotypes that vary in the concentrations of the principal terpenoids (Ireland, 1999; F. A. Dray and G. S. Wheeler, unpublished data), many of which are well-known mediators of mammalian (Lawler *et al.,* 1999) and invertebrate (Gershenzon and
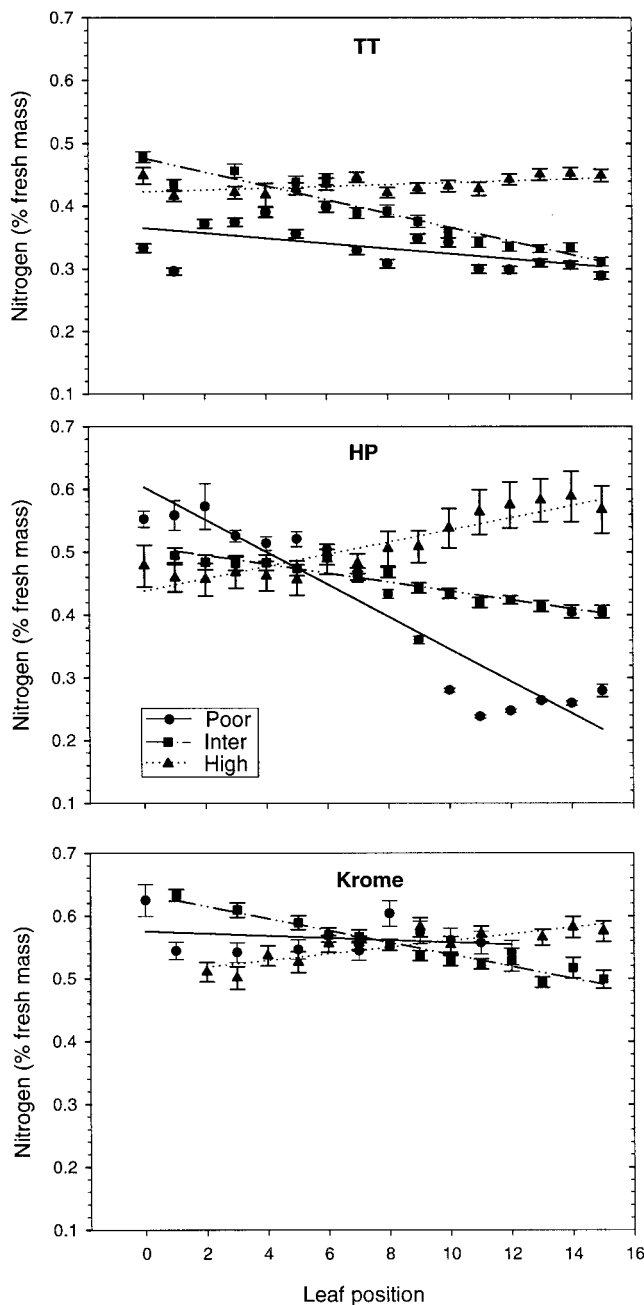
**FIG. 5.** Mean percentage nitrogen (fresh mass $\pm$ SE) of *M. quinquenervia* leaves collected at different leaf positions from the tip. Leaves were from three sites and three categories of plant quality from each site. Percentage nitrogen of leaves decreased in the poor-quality (see Table 2 for coefficients and probabilities) and intermediate-quality leaves and increased in the high-quality leaves from the branch tip toward its base.

Croteau, 1991) herbivore behavior, growth, and development. Examples include $\alpha$-pinene, a well-known factor that influences diverse insect–plant interactions (Gershenzon and Croteau, 1991), 1,8-cineole, an attractant of the banana weevil (Ndiege *et al.,* 1996) and

*E,S*-nerolidol, an antifeedant of the gypsy moth (Doskotch *et al.,* 1980). The distribution and biological significance of these and other *M. quinquenervia* terpenoids on *O. vitiosa* larval and adult performance and behavior need to be determined.

The classification of *M. quinquenervia* plant quality by leaf color (Anonymous, 1977) was proposed as a useful field estimate of relative plant nutrient levels and suitability for flush-feeding herbivores like *O. vitiosa*. In several cases, the technique accurately characterized the relative levels of percentage nitrogen of leaves and *O. vitiosa* larval survival and performance. For example at the TT site, percentage nitrogen was greatest for high-quality leaves, followed by intermediate-quality leaves, and was lowest for poor-quality leaves. Larvae fed the high-quality leaves from this site had their greatest survival, shortest development time, and greatest adult biomass, followed by the larvae fed the intermediate- and poor-quality leaves. Although larval survival and adult biomass did not differ significantly when the larvae were fed the intermediate- and poor-quality leaves from TT, development time was significantly greater for larvae fed the intermediate-quality compared with those fed the poor-quality leaves. However, the leaf quality classification system applied to leaves collected at the Krome site appeared to conflict with the percentage nitrogen determination as no difference was found in the latter values among the different leaf quality categories. However, larvae fed the high-quality leaves from the
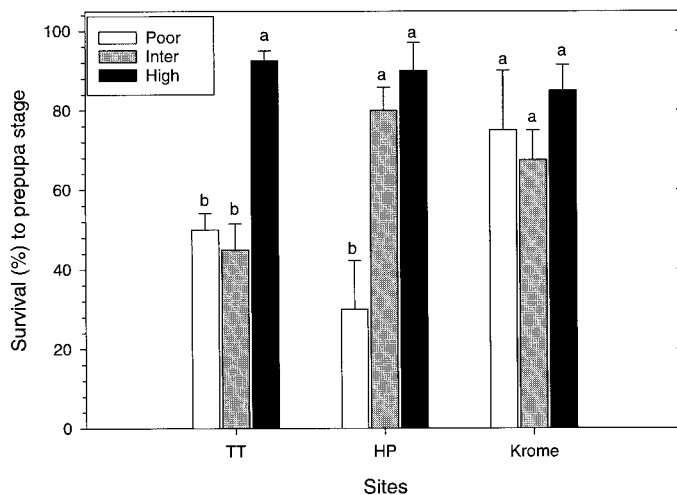


**FIG. 6.** Mean percentage survival ($\pm$ SE) of *O. vitiosa* larvae to the prepupal stage fed *M. quinquenervia* leaves from three sites and three categories of plant quality. No significant difference was found among sites. However, bars with different lowercase letters within a site were significantly different according to a Ryan's Q mean comparison test ($P < 0.05$). Percentage survival was significantly reduced in larvae fed the poor-quality and the intermediate-quality leaves from the TT site ($F_{2,9} = 31.65$; $P < 0.0001$) and the poor-quality leaves from the HP site ($F_{2,9} = 13.29$; $P = 0.0021$).
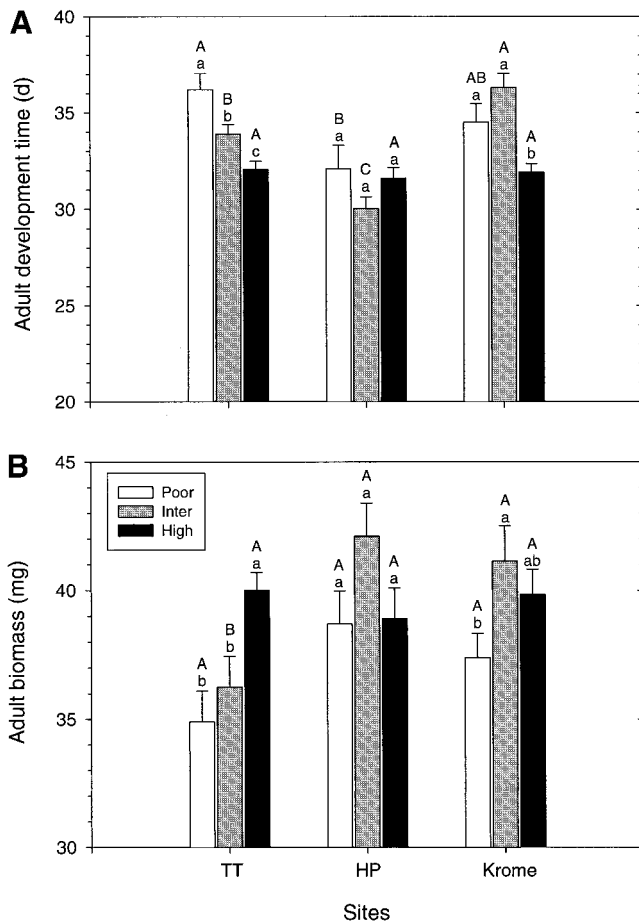
**FIG. 7.** Mean development time (± SE) from neonate to adult (A) and adult biomass (B) for *O. vitiosa* larvae fed *M. quinquenervia* leaves from three sites and three categories of plant quality. Bars with the same uppercase letters within a plant quality category were not significantly different and bars with the same lowercase letters within a site were not significantly different, both according to a Ryan's Q mean comparison test ($P < 0.05$).

Krome site completed development more rapidly than larvae fed leaves from the other leaf quality categories, and adult biomass was generally greater for larvae fed the high- and intermediate-quality leaves. Finally, for the HP site, only larval survival was greater for larvae fed the high- and intermediate-quality leaves compared with those fed the poor-quality leaves. Additional studies are needed to address other factors that may have influenced larval survival, growth, and development, such as the terpenoid levels of the leaves.

The larvae of *O. vitiosa* minimize the physical barriers of leaf toughness and nutritional deficiencies of limited water and nitrogen by feeding on the young leaves or flush growth of *M. quinquenervia* trees. The results of this study indicate that the apical leaves of branches with growing tips are softest and the leaf toughness increases toward the branch base. Additionally, the distribution of foliar percentage dry mass (moisture) and nitrogen from the branch tip toward its base was influenced by the quality of leaves. Percentage leaf dry mass was generally greatest, and so nutrients were less dilute, in leaves near the branch tip, especially in the intermediate- and poor-quality leaves, and this level generally decreased toward the branch base. Percentage nitrogen levels increased toward the base in the high-quality branches and decreased toward the base in the intermediate- and poor-quality branches. Eggs of *O. vitiosa* are generally laid singly or in small clusters on young leaves in or near the branch tip (Purcell and Balciunas, 1994). After eclosion, the larvae begin feeding on the same leaves near the branch tip. This oviposition behavior provides the neonates with the softest leaves on branches with growing tips. These leaves from the intermediate- and poor-quality branches also have the greatest percentage dry mass (lowest moisture) and nitrogen content. Possibly, *O. vitiosa* larvae are not water limited while feeding on these drier leaves, but, more importantly, essential nutrients such as nitrogen are more concentrated. Subsequently, larval development is completed on leaves farther from the tip with decreases in both percentage dry mass (greater moisture) and nitrogen for the intermediate- and poor-quality leaves with greater distance from the branch tip. On the high-quality branches the opposite occurs, where the leaves farthest from the tip have increased percentage dry mass and nitrogen. Possibly, *O. vitiosa* eggs and larvae are distributed similarly to take advantage of the nutritional differences on high- versus intermediate- and poor-quality branches. However intriguing, this relationship has yet to be determined. Most likely, the relatively high leaf toughness and its associated high *O. vitiosa* larval mortality have selected for oviposition, larval growth, and development on apical leaves of low toughness and often high nitrogen content.

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